

LXXII. THE EFFECTS OF VITAMIN-DEFICIENT DIETS ON THE ADRENALINE EQUILIBRIUM IN THE BODY.

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(A) INTRODUCTION.

IN 1919 McCarrison [1919] found that certain vitamin-deficient and otherwise unbalanced diets produced a marked increase in the size of the adrenal gland, which increase was associated with a larger store of adrenaline in the case of vitamin *B* deficiency. In fact he was at first inclined to believe that the appearance of oedema in the wet form of beriberi was to a large extent due to increased intracapillary pressure following on an increased output of adrenaline. In 1914 Funk and v. Schönborn [1914] found an increase in the blood sugar of pigeons fed on polished rice and in 1920 Funk [1920] suggested that it was due to increased adrenaline output. Anrep¹ observed in one or two cats which had been fed for a considerable time on vitamin *B*-deficient food that the blood-pressure was approximately 285 mm. Hg. while the animals were under anaesthesia. I have shown elsewhere² that deprivation of vitamin *B* from the diet of the adult rat leads almost at once to a pronounced intestinal stasis. These observations as well as the fact that adrenaline is a powerful gut inhibitor and that rats under advanced vitamin *B* deficiency show a number of signs resembling to some extent the effect of an increase in a sympathomimetic substance (*e.g.* nervousness, excitability, hair standing on end, etc.) made it seem very possible to me that the whole complex could be explained on a basis of hyperadrenalinemia [Gross, 1922]. Upon such a supposition, too, an explanation was afforded for the accumulation of large pigment-bearing cells confined to the subepithelial stroma of the colon which Keith [1914] described as occurring in advanced stasis in the human. For, granted that this pigment was taken in by phagocytes, or in some way obtained from the colon, (probably as a result of breaking down of aromatic decomposition

¹ Private communication.

² This will appear in a forthcoming publication.

products as suggested by Pick) these swollen pigment-bearing cells would find it impossible to pass lymphatics which were partially choked off by a muscularis mucosae kept in tonic contraction by increased adrenaline [Gunn and Underhill, 1914]. This explained, too, the lymphatic lakes and "soggy colon" of chronic intestinal stasis.

The experiments about to be described represent an attempt to confirm some of the statements made by these observers, and to determine directly the adrenaline content of the blood. 319 rats were used for the purpose.

(B) THE RELATION OF BODY WEIGHT TO ADRENAL GLAND
WEIGHT AND ADRENALINE STORE.

The greater number of observations published on this subject are based on adrenal glands taken from animals which were either dead or dying. Furthermore, the diets largely used were grossly unbalanced so that the results in any case cannot be held to be those following pure vitamin deficiencies. The question seemed therefore worth reinvestigating in order to determine the changes in these glands which follow pure vitamin deficiencies on otherwise well balanced diets, and to make the adrenaline determinations on glands from adult rats which show evidences of vitamin deprivation, but are, on the whole, still in a relatively fair condition of health.

Table I.

Table of the diets used, showing the proportions of the ingredients in parts by weight. The butter was mixed with 2 % of its bulk of cod-liver oil. The heated casein was prepared by heating shallow layers of casein in a dry oven for 24 hours at a temperature of 110° C. The extracted casein was prepared by extracting casein with boiling 95 % alcohol for 4 hours. "Marmite" is a commercial preparation of autolysed yeast. The hardened fat used was hydrogenated cotton seed oil. The salt mixture was as follows in parts by weight:

	Sodium chloride	5.19		Calcium lactate	39.00		
	Magnesium sulphate	7.98		Ferric citrate	3.54		
	Sodium acid phosphate	10.41		Calcium phosphate	16.20		
	Potassium phosphate	28.62					
Diet	Casein	Starch	Hardened fat, or	Salt mixture	Marmite	Lemon juice	Water
	20 parts	50 parts	butter, 10 parts	5 parts	5 parts	5 parts	30 parts
<i>P</i>	Casein	"	Butter	"	"	"	"
<i>A</i>	Heated	"	Hardened fat	"	"	"	"
<i>B</i>	Extracted	"	Butter	"	None	"	"
<i>C</i>	Casein	"	Butter	"	Marmite	None	"

The diets used in these experiments are shown in Table I. The average time during which the rats were kept on the various diets, as well as their average weights at the beginning and end of the experiment, are shown in Table II.

The rats were killed by a sharp blow on the head with a metal rod immediately followed by amputation of a fore limb. The blood was caught in a clean glass receptacle to be subsequently used for various experiments. This method of killing, besides being even more humane than coal-gas poisoning, gives an excellent yield of clean uncontaminated blood. The tissues are of course unaltered for histological purposes.

Table II.

The rats on *P* diet showed a steady increase in weight. The rats on *B* diet showed a consistent fall in weight. The rats on *A* and *C* diets presented a flattened growth curve which was more exaggerated in the case of *A* diet. The figures which give the average weights at the end of the experiment in this table include an average final fall in weight of 10 g. in the case of *A* diet, and an average final fall of 8 g. in the case of *C* diet.

	<i>P</i> diet	<i>A</i> diet	<i>B</i> diet	<i>C</i> diet
Average time on diet, in weeks	8	6	5	7
Average weight at beginning of experiment	184	205	228	193
Average weight at end of experiment	219	207	194	204

The method employed for estimating the adrenaline content of the adrenal glands was that of Folin, Cannon and Dennis [1912], which was largely used and found quite satisfactory by McCarrison [1921] and by Kellaway [1920]. The only modification which I adopted was to add the uric acid solution to my adrenaline extract in a quantity equal to that used in the standard tube. In this way the estimation of the small quantities of adrenaline found in a rat's adrenal glands was made possible. I found that the end-point in the colorimetric readings could be made considerably sharper by interposing an orange-coloured screen between the mirror of the colorimeter and the source of light. The latter should preferably be artificial. I used an Osram Daylight bulb, employing this light reflected from milk glass. The adrenal glands were obtained immediately after death and carefully dissected free from the surrounding fat. They were then weighed and the adrenaline estimated.

The results in this investigation are the averages from 350 glands obtained from 175 rats. A number of mice were also employed, but the experimental error was found too great to render these observations of any importance. Table III has been constructed entirely on the figures from rats' glands.

Table III.

	<i>P</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>B</i> deficiency followed by <i>P</i> diet
(a)	0.0253	0.0221	0.0188	0.0194	0.0174
(b)	0.0253	0.0228	0.0217	0.0208	0.0174
(c)	1.75	1.82	2.03	1.71	3.88
(d)	0.0442	0.0403	0.0382	0.0332	0.0675
(e)	0.0442	0.0415	0.0440	0.0356	0.0675

(a) represents g. of adrenal gland per 100 g. of highest body weight of rat.

(b) represents g. of adrenal gland per 100 g. of final body weight of rat.

(c) represents milligrams of adrenaline per g. of adrenal gland.

(d) represents milligrams of adrenaline per 100 g. of highest body weight of rat.

(e) represents milligrams of adrenaline per 100 g. of final body weight of rat.

Row (a) in Table III shows the comparison in weight between the glands of normal and vitamin-deficient rats. The numbers are all reduced to grams of gland per 100 g. of highest body weight of rat. The first fact to be noticed is that the adrenal glands of rats on the different vitamin-deficient diets decrease in size. This is most marked in vitamin *B* deficiency and least in vitamin *A* deficiency. Rondoni [1914, 1915], McCarrison [1919] and others [see McCarrison, 1921] have shown that vitamin and other dietary deficiencies cause a considerable increase in the size of the adrenal glands. McCarrison

conducted his experiments on monkeys, guinea-pigs and pigeons. My figures indicate the complete opposite for adult rats on pure vitamin deficiencies. The difference must depend either on the different species used, or the unbalanced diets used by these observers, or on changes during the last stages of vitamin deficiencies. Very likely the second reason given is the most important. I have noticed that the adrenal glands appeared noticeably enlarged and congested in several rats which I had deliberately kept on vitamin *B* deficiency to the point of death. The last stages of vitamin *B* deficiency are, however, undoubtedly complicated by the results of inanition. The average duration of my rats on vitamin *B*-deficient diet from which these figures are taken, is 5 weeks. One other point is worth noticing. In the normal rat, the adrenal gland weight is not in linear proportion to body weight. The heavier rats have as a rule slightly smaller adrenals per 100 g. of body weight than have the lighter or smaller rats. Attention must be paid to this point in comparing the effects of the various experimental diets. Table II shows that there is comparatively little difference between the average weights of my animals.

Row (*b*) represents grams of adrenal weight computed per 100 g. of final weight of rat. These figures show that the atrophy of the adrenal gland is not only absolute but relative as well.

Row (*c*) represents milligrams of adrenaline per g. of adrenal gland. It is seen that the adrenaline content of the adrenal glands of vitamin *B*-deficient rats shows a moderate relative increase in the store of adrenaline per weight of gland as compared to the content of adrenal glands from normal, *A*- and *C*-deficient animals. When, however, the gland content of adrenaline is reckoned per 100 g. of highest body weight of rat (row (*d*)), it is seen that an absolute diminution has taken place in vitamin *B*-deficient rats and even more so in *C*-deficient rats. Finally, when the adrenaline in the glands is computed against final body weight, which is nearest to the relation which it had during the life of the rat while on the experimental diet, it is seen that even these differences largely disappear (row (*e*)). *C* diet is the only deficiency which appears to produce any appreciable change. This change, however, is of the order of 19 %, and in view of the relatively high technical errors involved in determinations of this kind, too much significance must not be attached to it.

An entirely unexpected result was the relatively small glands found in rats which had been on a vitamin *B*-deficient diet for an average of 5 weeks and then placed on normal diet until the normal weight had been regained (usually about $2\frac{1}{2}$ weeks). The average weight of gland per 100 g. of rat was 0.0174 g. The adrenal content was extremely high, 3.88 mg. per g. of gland. Worked out in term of mg. of adrenaline per 100 g. of highest weight of rat which was in this case equal to final weight, we arrive at the astonishing figure of 0.0675. This represents an absolute increase of 53 %.

Other points brought out were: (*a*) Unilateral adrenalectomised rats showed an increase in the store of adrenaline in the remaining gland. Thus,

whereas the normal gland showed 1.75 mg. the gland remaining after unilateral adrenalectomy contained 2.34 mg. of adrenaline per g. of gland. (b) Daily injections of adrenaline seemed to discharge, or perhaps decrease the formation of adrenaline in, the glands to a moderate extent, causing them to contain approximately 13 % less than on the same diet without the adrenaline injections. This was true for both normal and vitamin *B*-deficient diets.

Microscopic examination of the adrenal glands from normal rats as well as from those deficient in vitamins *A*, *B* and *C* respectively showed no appreciable change. The fixatives and stains used were 10 % neutralised formalin in saline, and osmic vapour [Cramer, 1918]. This investigation therefore shows that if the diet is otherwise well balanced, and if the adult rat is not kept on this diet until moribund, the changes in the adrenal glands both anatomically and functionally are comparatively slight.

(C) ATTEMPTS TO DETERMINE THE AMOUNT OF ADRENALINE PRESENT
IN THE BLOOD AND SERUM OF NORMAL AND VITAMIN-DEFICIENT RATS.

This investigation was carried on at the same time, and on the same animals, as were used for the experiments described in the preceding section. It was not until the completion of the latter experiments that the statistical evidence derived therefrom pointed to the fact that when the store of adrenaline is compared to the weight of the animal, it is found that pure vitamin deficiencies produce on the whole very little, if any, disturbance in the adult rat. When the studies about to be described were being carried out, therefore, it was still on the assumption that vitamin deficiencies cause an upset in the adrenaline equilibrium with probably a resultant hyperadrenalinemia in the case of vitamin *B*.

The methods which I employed were as follows:

(a) Effect of serum on the isolated frog's pupil.

(b) Effect of blood and its derivatives on various other isolated organs such as rat's and rabbit's uterus and intestine, perfused rabbit's ear and perfused frog. In this study I co-operated with Professor A. J. Clark [unpublished].

It is, unfortunately, impossible to isolate adrenaline from the blood at present. Attempts at ultrafiltration and subsequent precipitation resulted in failure, I therefore turned my attention first to the use of the isolated frog's pupil. This method, which was first suggested by Meltzer and Auer [1904] and of which a critical survey was made by Schultz [1909], is not a specific test for the presence of adrenaline, for the mydriasis caused by this substance can also be brought about by other substances probably normally present in blood, *e.g.* pituitary extract. Nevertheless, in the hope that some idea of the relative amounts of mydriatic substance present in the serum would furnish useful evidence on the question, 63 rats were used for this purpose.

The blood was obtained in the manner already described. It was received

into a centrifuge tube and immediately spun for 5 minutes at 4000 revolutions per minute. The separated serum was pipetted off and 0.35 cc. run into each of several dwarf test-tubes. Frogs had meanwhile been pithed, the eyeballs carefully removed and placed upon a sheet of white paper with the pupils facing a constant source of light. It was planned to expose these pupils to the light for 5 minutes. The two diameters of the frog's ellipsoidal pupil were spanned with a pair of tool-maker's dividers and measured on a Vernier scale. The eyeballs were then immersed, one in each tube of serum. The same measurements were taken a half-hour later and one hour later, care being taken to carry out the whole process under similar conditions.

The areas of the pupils were computed from the measurements obtained, and the mydriatic effect of the serum expressed in terms of per cent. of the area increase over that found in the pupil just before immersion into serum. For controls, sera from normal rats were used.

A separate series of control experiments had brought out the following points:

(a) There was a well-marked variation in the reaction of the different frogs' pupils to the same serum, sometimes even the two eyes of the same frog gave a different reading for the same serum.

(b) On the whole the average of a fair number of eyes gave figures for known concentrations of adrenaline added to saline, which showed a distinct diminution in mydriatic power with increased dilution of the adrenaline. The utmost sensitivity, which I could obtain by this method, was a reaction to 1 in 8,000,000 adrenaline hydrochloride in saline.

Table IV.

Per cent. of area increase in frog's pupil: (a) after one half hour immersion in serum; (b) after one hour immersion.

	<i>P</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>B</i> deficiency followed by <i>P</i> diet
(a)	39	23	75	42	114
(b)	25	29	49	22	90

Table IV shows the results of these tests. It is seen that the serum of vitamin *A*-deficient rats has a somewhat lower mydriatic power, and that of vitamin *B*-deficient rats a considerably higher power, as compared to the controls. Also *B* rats which were subsequently fed on *P* diet for about two weeks showed an even greater mydriatic power of the serum. It will be remembered that the adrenaline content of these glands was considerably increased. This, in fact, is the only relation which I could find between the mydriatic power of the serum and the adrenaline store in the glands. Other points not shown in the table were the considerable decrease in mydriatic power of serum from unilateral adrenalectomised rats. The serum from rats which had daily injections of adrenaline showed neither in control nor in vitamin *B*-deficient rats, a departure from that found in the normal. In view

of the individual variations, the latter experiments as well as those on vitamin *A*-deficient animals, were rather too few in number to warrant more than a possible suggestion for future investigation.

It is seen, therefore, that if this increase in mydriatic power in the serum from vitamin *B*-deficient rats is due to adrenaline, the concentration in the serum should be well over 1 in 8,000,000.

To test this, Professor Clark and I compared the effects of fresh blood within 20 seconds from being shed, defibrinated blood, serum, plasma, ultrafiltrate of serum and plasma, and alcoholic and ether extracts of serum and plasma on the isolated organs previously mentioned in this section. Considering the fact that some of the organs, for example, rat's uterus, were occasionally sensitive to 1 in 500,000,000 adrenaline, well-marked reactions should have been obtained. It is true that shed blood very quickly develops vaso-constrictor and smooth muscle stimulating substances, but we endeavoured to obtain at least a quantitative difference between the blood of normal and vitamin *B*-deficient animals which would indicate a higher content of adrenaline in the blood of one of these groups.

Our results, which are described in greater detail in a separate publication, merely demonstrated the enormous variability of these fluids and tissues. We were, in short, unable to establish any definite differences between the adrenaline content of the blood of the normal and vitamin-deficient animals. Nor were we able to find any other characteristic differences in the properties of the blood of these animals in their effects on the isolated organs tested. It seems, therefore, highly improbable that the increased mydriatic effect of the serum which I found in vitamin *B*-deficient rats was due to a higher concentration of adrenaline.

(D) INVESTIGATIONS ON THE CARBOHYDRATE METABOLISM OF VITAMIN *B*-DEFICIENT RATS.

The chief interest which I had in the question was in so far as it would furnish indirect evidence on the relative amount of adrenaline in the circulation. Mr P. Eggleton and I are, however, making a detailed study of the carbohydrate metabolism in *B*-deficient rats which we hope will be published shortly. The present preliminary notice is based on the results from 48 rats. On the whole these are negative.

In 1914 Funk [1914] suggested that the anti-beriberi vitamin plays some part in carbohydrate metabolism. He based this view upon the fact that addition of carbohydrate to a standard beriberi diet caused the symptoms to appear earlier in pigeons. In the same year Funk and v. Schönborn [1914] found supporting evidence for this hypothesis in the discovery of a hyperglycemia and low liver glycogen in beriberi pigeons. They also observed that the administration of yeast tended to lower the blood sugar and increase the store of glycogen in the liver. In criticising these results, Vedder [1918] pointed out that overfeeding was probably the determining factor. In 1919

McCarrison [1919] stated that the absence of "anti-neuritic" food factors from the diet leads to an increase in the weight and adrenaline load of the adrenal gland and to a state of acidosis due to the imperfect metabolism of carbohydrates and the acid fermentation of starches. How far the increased weight of the adrenal gland and increased adrenaline store is due to deprivation of vitamin *B*, and how much to inanition, has been discussed in section (B). On the basis of McCarrison's results, Funk [1920] pointed out that increased adrenaline in the circulation was probably responsible for the hyperglycemia which he had obtained in another series of beriberi pigeons (bled out under A.C.E. mixture). It is noteworthy that Funk's figures show in this experiment a higher glycogen content in the beriberi livers than is seen in his normal controls. This is, of course, the opposite to what he and v. Schönborn had previously found. Very recently Mattill [1923] studied the respiratory quotient of vitamin *B*-deficient rats and concluded that there was no interference with the process of glucose combustion.

This condensed review presents the chief points brought forward which bear on the relation between vitamin *B* and carbohydrate metabolism. It was obviously important to reinvestigate this question in order to determine:

(a) Is pure vitamin *B* deficiency in the rat associated with an alteration in the carbohydrate metabolism?

(b) If so, what part in the cycle is affected, and in what manner?

The method which we employed was as follows: a large number of rats were fed on vitamin *B*-deficient diet and at different stages of the deficiency some were used for blood sugar and liver glycogen determinations. Experiments were also made on the blood sugar following the digestion of starch and that following the absorption of glucose. Since it is very difficult to obtain more than one sample of blood sugar from a rat without having recourse to anaesthesia, we killed the rat in each instance and relied upon statistical evidence of numbers for our conclusions. This eliminated most of the artefact which would otherwise be introduced into the actual sugar values. Also, much of the individual variation was thereby brought to a more reliable average. For blood sugar determinations we employed the MacLean [1919] 0.2 cc. method which we found quite reliable. For glycogen determinations we used Pflüger's method [1910], finally determining the hydrolysed sugar by Bertrand's method [1920].

The points investigated were as follows:

(a) *Blood sugar and liver glycogen from normal and vitamin B-deficient rats as they were taken out of the cage.* This is of course open to the objection that the blood sugar will depend upon when the animal had its last meal. These determinations were made, however, in order to compare the results with some work published by other authors who do not mention the precaution of preliminary starvation. The results obtained showed no appreciable difference for blood sugar, but a considerably lower liver glycogen in the *B*-deficient rats.

(b) *Blood sugar and liver glycogen from rats starved for 24 hours.* This is much more reliable since it does away practically entirely with carbohydrate absorption from the bowel. The liver glycogen in both normal and vitamin B rats was nil. The blood sugar was slightly higher in the B-rats than in the normal (0.09 g. % for normal, 0.117 g. % for B). It is possible, however, that additional numbers will reduce or remove this difference.

(c) *Rate of absorption of glucose from the alimentary tract of rats starved for 24 hours.* A glucose solution of known strength was introduced by catheter into the stomach, and blood sugar estimations were made at 15-minute intervals for one hour. No significant differences were found in the blood sugar curves from both groups of animals.

(d) *Introduction of a starch paste* gave us an indication of the rate of digestion as well as of absorption. Again no appreciable differences were found between normal and vitamin B-deficient rats.

In none of these experiments was a glycosuria found.

It is seen, therefore, that there is not as yet sufficient evidence to attribute to vitamin B deficiency an upset of carbohydrate metabolism, at any rate of the part studied by us thus far. Certainly the changes which we have found are insufficient to account for the marked general disturbances of the animal and do not afford adequate confirmatory support to the theory of hyperadrenalinemia. It is possible, however, that larger numbers of rats may reveal evidence which has not thus far been brought out.

(E) CONCLUSIONS.

The general conclusion which can be drawn from these investigations is that pure vitamin deficiencies set up very little alteration in the adrenaline equilibrium (*i.e.* relations between adrenaline store, gland weight and body weight) in the adult rat, providing the rat is not brought to the point of death on the diet. Such alterations as do occur can be explained on a basis of general tissue atrophy. The striking changes which have been recorded by other observers have probably been produced by the accompanying starvation and unbalanced dietary rather than by the deficiency in vitamins. Unless indeed, species so widely different as pigeons, guinea-pigs and monkeys are predisposed to alterations in adrenaline equilibrium to a much greater degree than is the adult rat. This seems very unlikely. Contrary to an upset in adrenaline equilibrium, the adrenaline store in the adrenal gland maintains an extraordinarily constant proportion to the weight of the rat. The suggestion forces itself upon one that this is no mere coincidence and that the remarkable way in which the store of adrenaline follows the body weight in its vicissitudes caused by the vitamin deficiencies argues very strongly for a finely controlled mechanism. It is then perhaps not surprising that I have been unable to obtain either direct or indirect supporting evidence of an unmistakable alteration in the adrenaline content of the blood.

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